

## IN THE CLAIMS

Please amend Claims 1 and 97 as follows. Claims 2-4, 6-13, 15-22, 24-26, 28-29, 31-33, 35-39, 41-56 and 58-83 have been previously cancelled. Claims 1, 5, 14, 23, 27, 30, 34, 40, 57 and 84-110 are currently pending<sup>1</sup> of which Claims 27, 30, 34 and 57 have been withdrawn from consideration. The listing of claims below will replace all prior versions, and listings, of claims in the Application:

1. (Currently Amended) Continuous multi-microencapsulation process of biologically active materials by means of in situ interfacial polymerization characterized in that the process is performed under continuous agitation and comprises the following steps:

(a) in a first step a water phase is emulsified into an oil phase; wherein

a.1. a polymerization initiator exists in the water phase,

a.2. an emulsifier exists in the oil or in the water phase,

a.3. at least a biologically active ingredient exists in the oil and/or in the water phase;

(b) in a second step, a solution or dispersion in water that contains at least one hydrocolloid is added to the emulsion, wherein the hydrocolloid is polymerizable due to the polymerization initiator, this producing a phase inversion and the polymerization and cross-linking of the polymerizable hydrocolloid(s) onto the water in oil droplets;

(c) in a third step, a solution or dispersion in water that contains at least one protective colloid is added that begins to be deposited on the surface of the drops of water in oil, and to polymerize and cross-link with itself and the ~~hydrocolloid~~; hydrocolloid;

(d) in a fourth step, a solution or dispersion in water of a surfactant is added to allow a reduction of the size of the water in oil ~~drops~~; drops;

(e) in a fifth step, during the process of reduction of size, the partially formed microcapsules are deagglomerated and reagglomerated, such that an enclosure of drops inside bigger drops eventually happens; and

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<sup>1</sup> The Office Action Summary incorrectly does not list Claims 107-110 as currently pending.

(f) when enough time has passed in order that the oil and/or water in oil drops are covered by at least one hydrocolloid and at least one protective colloid, the temperature is increased in order to strengthen the wall of the formed microcapsules or multi-microcapsules suspended in water.

2-4. (Cancelled)

5. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the hydrocolloid(s) of the second step and protective colloids of the third step are chosen from the group: chitosans, starch, dextrans, cyclodextrins, celluloses, pectines, agar, alginates, carrageens, gelatins, seed gums, xantan gum, guar gum, acacia gum, arabic gum, *Caraya* gum, *Cerantionia siliqua* gum, *Pysllium* gum, gelatin, tragacanth, lignin, lignosulfonates, saponines, galactomanans, arabanogalactams, beta-glucans, inulin; in all their isomeric and stereochemical forms, in all their variations regarding quantity and proportion of monomers or oligomers constituting the hydrocolloid, in their natural or derivatized forms, and as their salts of metal cations or nitrogenated, sulfurated or phosphorinated derivatives, albumin, polyarboxylates, poly-L-lactid.

6-13. (Cancelled)

14. (Previously Presented) Process of microencapsulation of biologically active materials according to claim 1, characterized in that the aqueous solution of hydrocolloid contains a binary or ternary mixture of the hydrocolloids.

15-22. (Cancelled)

23. (Previously Presented) Process of microencapsulation according to claim 1, characterized in that after the drying of the microcapsules, these are reformulated and dispersed in an oil phase or in a gel or in any semi-solid material or ethanolic solution or organic solvent.

24-26. (Cancelled)

27. (Withdrawn) Microcapsules produced by a continuous process of microencapsulation, characterized in that:

- (a) contain biologically active materials;
- (b) the microcapsules wall is made by a mixture of at least two hydrocolloids (including hydrogels as particular case of hydrocolloids), such mixture polymerized and cross-linked,
- (c) the polymerization and cross-linking grade and the nature of hydrocolloids influence the release rate and the protection against oxygen and/or light and/or temperature,
- (d) the microcapsules have in their core an emulsion water in oil, existing optionally biologically active materials in the oil phase, optionally in the water phases and optionally in all continuous phases, and moreover, the core of the microcapsules may contain smaller microcapsules (multi-microencapsulation possible at least to five degrees),
- (e) the mean particle size measured with a Master Sizer type laser equipment is 0.1-100 urn, preferably 1-10 urn,
- (f) at least a biologically active ingredient is present in at least a discontinuous oil phase and/or in a discontinuous water phase they are produced by a continuous process of multi-microencapsulation process by interfacial in-situ polymerization process.

28-29. (Cancelled)

30. (Withdrawn) Microcapsules according claim 27 characterized in that they are used for providing anabolites and/or nutrients in microbiological cultures in a constant or quasi-constant rate.

31-33. (Cancelled)

34. (Withdrawn) Microcapsules according claim 27 characterized in that they are used for providing beneficial for the health materials and the microcapsules are added to natural or synthetic sweeteners, salt, pepper, spices and other condiments, in such a way that the addition of such condiments to other foodstuffs increment the nutritive value or the health benefit of such foodstuffs.

35-39. (Cancelled)

40. (Previously Presented) Process of microencapsulation of biologically active materials according to claim 1, characterized in that at least one of the biologically active materials present

in the formulation consist in probiotic bacteria, optionally acid lactic-bacteria and more preferably chosen among the group: *Lactobacillus casei*., *L. acidophilus*, *L. rhamnosus*, *L. paracasei*, *L. gasseri*, *L. fermentum*, *L. plantarum*, *L. salivarius*, *L. crispatus*, *L. bulgaricus*, *L. fermentum*, *L. reuteri*, *Bifidobacterium infantis*, *B. bifidum*, *Streptococcus thermophilus*, *S. bovis*, *Enterococcus durans*, *E. faecalis*, *E. Gallinarum*, *Escherichia coli*, *Propionibacterium freudenreichii*, or bacteria or fungi or yeasts genetically modified in that the beneficial genes - characterizing the beneficial properties of probiotic bacteria- have been inserted.

41-56. (Cancelled)

57. (Withdrawn) Microcapsules produced according to claim 27, characterized in that they are stable (no opening of the microcapsule's wall) at pH higher than 3.5.

58-83. (Cancelled)

84. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that it is carried out under reduced pressure.

85. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that it is carried out in the presence of an inert gas.

86. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that it is carried out protected from visible or ultraviolet light.

87. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the emulsions and reduction of particle size are performed at an agitation speed of 3000 to 25000 rpm.

88. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the size of the droplets of the emulsion of the first step is of 50-500  $\mu\text{m}$ .

89. (Previously Presented) Process of microencapsulation according to claim 88 characterized in that the size of the droplets of the emulsion of the first step is 70-200  $\mu\text{m}$ .

90. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the hydrocolloid of the second step and the protective colloid(s) of the third step are added together in the form of an aqueous solution or dispersion.
91. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the protective colloid(s) belong to the chemical group of hydrocolloids.
92. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the oil phase is comprised of an hydrogenated oil or a wax or honey.
93. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that one of the emulsifiers used is based in soya containing compounds.
94. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that one of the emulsifiers added in the fourth step is a glyceride ester derivative.
95. (Previously Presented) Process of microencapsulation according to claim 1 characterized in the emulsifiers added in step is a glycerol ester of tartaric acid.
96. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the emulsifier used in the fourth step has a HLB of 12-14.
97. (Currently Amended) Process of microencapsulation according to claim 1 characterized in that the ~~viscosity modifier is a~~ hydrocolloid xanthan gum is added at any stage of the process wherein a hydrocolloid is used.
98. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the hydrocollolds used in second step are of the type of alginates.
99. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the protective colloid is arabic gum.
100. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that it is added a further biologically active ingredient in any step of the process, in the form of a solution, dispersion or emulsion.

101. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the water phases contain at the most 40% of an alcohol of molecular weight up to 144 units of atomic mass.

102. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the oil phase consists in fish oil with omega-3 fatty acids or in an arachidonic acid enriched oil or in conjugated linoleic acids.

103. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the oil phase consists in a vegetable oil extract of flax oil or *Borago spp.*

104. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the hydrocolloids used for forming the wall, allow the release of the content of the microcapsules at pH lower than 3.

105. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the oil phase contains vitamin E or ascorbyl palmitate and at least one water phase contains ascorbic acid.

106. (Previously Presented) Process of microencapsulation according to claim 1 for its use in production of foodstuffs enriched with biologically active materials, characterized in that:

(a) the process is kept at about 30-70 °C until the finalization of the polymerization and cross-linking reactions and then the temperature is raised to about 60-100 °C in order to cure the microcapsules;

(b) the final microcapsules have an average size of about 1-30 µm;

(c) after the curing step, it is added a food-grade viscosity modifier;

(d) during the process only food-grade emulsifiers are used.

107. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that it is added an additional step of microbiological stabilization by means of pasteurization, UHT, sterilization, ozonization, ultraviolet light or gamma rays irradiation or addition of antimicrobial chemical agents.

108. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that an additional drying step is made at the end of the process in order to obtain dried microcapsules in the form of powder.

109. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that at the end of the process, the resulting suspension of microcapsules in water is lyophilized or spray dried.

110. (Previously Presented) Process of microencapsulation according to claim 1 characterized in that the biologically active material(s) is(are) selected according any of those mentioned in the preceding claims.